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# Microcystin in Missouri reservoirs

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## Abstract

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During summers (May–Aug) 2004–2006, 177 Missouri reservoirs were sampled monthly at open pelagic locations to assess regional patterns in microcystin concentration, frequency of occurrence over successive summer seasons and relations with environmental factors. Microcystin was detected in 58% of Missouri reservoirs and 23% of samples (n = 1402). Total microcystin concentrations, measured by enzyme-linked immunosorbent assay, ranged from ≤ 0.1 to 21 μg/L. Concentrations ≥ 1 μg/L were detected in 10% of reservoirs and exceeded the human health concern limit of 20 μg/L once in a single sample. Microcystin occurred throughout summer, with maximum concentrations in individual reservoirs observed in each month. Occurrence was consistent across years, with about one-half of Missouri reservoirs having detectable microcystin each summer. Eleven reservoirs with microcystin maxima ≥1 μg/L were sampled multiple seasons; of these, 8 had detectable microcystin each summer, which indicates that short-term surveys can identify water bodies with the greatest potential for toxin production. Eutrophic reservoirs in northern Missouri had the greatest microcystin occurrence and concentrations. Reservoirs with detectable microcystin had significantly ($p < 0.01$) greater nutrient and chlorophyll values and significantly shallower Secchi depths than reservoirs without detection. All correlations, however, had r-values ≤ 0.35, and bivariate plots indicated nonlinear trends. Cylindrospermopsin was measured by enzyme-linked immunosorbent assay in 36 reservoirs once in late summer 2006; 14% had small detectable levels (total concentrations <1 μg/L). This is the first report of cylindrospermopsin in Missouri.

Key words: cyanobacteria, cylindrospermopsin, microcystin, Missouri reservoirs, toxin



Microcystins (MC) are a class of cyanobacterial hepatotoxins common in lakes and reservoirs worldwide (Chorus and Bartram 1999, Huisman *et al.* 2005). Regional studies indicate MC occurs frequently in the Midwestern United States and may reach concentrations of human health concern (McDermott *et al.* 1995, Dodds 1996, Graham *et al.* 2004, Hedman *et al.* 2008, Lindon and Heiskary 2008). Whereas several studies in the United States have assessed regional patterns in MC occurrence (McDermott *et al.* 1995, Dodds 1996, Zimba and Grimm 2003, Graham *et al.* 2004, Boyer 2007, Williams *et al.* 2007, Hedman *et al.* 2008, Lindon and Heiskary 2008), few have evaluated frequency of occurrence during successive summer seasons. Likewise, few regional studies have developed empirical relations between environmental factors and MC concentration. Knowledge of MC occurrence during summer and the general environmental conditions associated with elevated concentrations will improve understanding of factors favoring toxic blooms.

Most Missouri reservoirs are located in the agricultural Osage and Glacial Plains, the Ozark Highlands, which are covered by forest and pasture, or the ecotonal Ozark Border section (Jones *et al.* 2008a). Reservoir nutrients are affected by hydraulic flushing and depth but exhibit an increase with cropland and a decrease with forest cover (Jones *et al.* 2004, 2008b). An initial survey of particulate MC (net collections >64 μm) in Missouri reservoirs indicated MC occurrence was more common in the eutrophic Osage and Glacial Plains reservoirs than those in the Ozark Highlands (Graham *et al.* 2004). A subsequent study of MC in the size fractions of natural plankton communities indicated particulate MC underestimates total toxin concentration because of algal loss in net collections (Graham and Jones 2007). This study reports total MC concentrations in whole water samples collected in conjunction with a statewide inventory

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of 177 Missouri reservoirs during summers 2004–2006 (Jones *et al.* 2008a). Data on the occurrence and concentration of MC are analyzed to describe regional patterns, detection frequency across three summer seasons and relations with reservoir nutrients and transparency. Also, cylindrospermopsin, another cyanobacterial hepatotoxin (Chorus and Bartram 1999), was measured in 36 reservoirs once in late summer 2006 to gather preliminary data on its occurrence; algal taxa capable of producing this toxin (*Anabaena*, *Aphanizomenon* and *Cylindrospermopsis*) can dominate summer algal assemblages in Missouri reservoirs (Graham *et al.* 2006a, Jones *et al.* 2008a). This information can facilitate human health protection in lakes and reservoirs used for recreational activities and drinking-water supplies.

# Methods

## *Sample collection*

Missouri reservoirs (n = 177) were sampled May through August 2004–2006: 94 were sampled one summer season; 45 were sampled 2 summers; and 38 were sampled all 3 summers. Most reservoirs were sampled monthly (n = 3 or 4 per summer), with individual reservoirs sampled on 3–26 occasions during this study. The reservoirs encompassed the full range of landscape, morphological and limnological conditions typical of Missouri reservoirs (Table 1; Jones *et al.* 2004, 2008a, 2008b).

Temperature, dissolved oxygen and Secchi transparency were measured at open pelagic locations in each reservoir, typically near the dam. Water samples were composited from the surface layer (0.25–0.5 m) and analyzed for total MC, conductivity (μS), turbidity (NTU), chlorophyll (Chl; uncorrected for degradation products), total phosphorus (TP), total nitrogen (TN), non-volatile suspended solids (NVSS), volatile suspended solids (VSS), total suspended solids (TSS), and dissolved organic carbon (DOC). Whole-water samples underwent 3 freeze-thaw cycles after Graham and Jones (2007) to lyse cyanobacterial cells and allow determination of total MC. In 2004, Envirogard® enzyme-linked immunosorbent assay (ELISA) kits (detection limit 0.1 μg/L; kit is cross-reactive with microcystin-LR, -YR, and -RR and nodularin) were used to measure MC. During 2005–2006, Abraxis® ELISA kits were used (detection limit 0.1 μg/L; -add a specific). Both kits provided similar results, and among-reservoir variability was greater than among-kit variability (J. Graham, unpublished data). Total cylindrospermopsin (CYL) was measured in 36 Missouri reservoirs once in late summer 2006 using Abraxis® ELISA kits (detection limit 0.04 μg/L). DOC was determined colorimetrically (Technicon Method No. 451–76W and Ontario Ministry of the Environment document JC 7501, 1972). Details of other analytical methods and land-use data are presented elsewhere (Knowlton and Jones 1995, Graham *et al.* 2004, Graham and Jones 2007, Jones *et al.* 2008a, 2008b).

## *Statistical analyses*

Reservoir trophic status was determined using geometric means (n = 3–18 per summer), which were averaged across all seasonal means (n = 1–3), and criteria for Missouri reservoirs (Jones *et al.* 2008a). Summary statistics were calculated using all available data; where MC was not detected, values of 0.05 μg/L (one-half the method detection limit) were substituted (Sokal and Rohlf 1995). All data were grouped into 3 MC categories based on concentration: no detectable MC; MC between 0.1–1 μg/L; and MC > 1 μg/L. Detections of large MC concentrations were too few to allow creation of additional MC categories above 1 μg/L. Significant differences in environmental variables associated with each MC category were determined using one-way analysis of variance (ANOVA) followed by Tukey's pairwise comparisons (Sokal and Rohlf 1995). Relations between total MC concentration and environmental variables were developed using Pearson correlation analysis (Sokal and Rohlf, 1995). All data were $\log_{10}$ transformed (land-use data were logit transformed) for ANOVA and correlation analyses (Sokal and Rohlf 1995). Statistical analyses were performed using SAS® (9.1), and significance was set at $p < 0.05$ for all analyses.

Bivariate plots of the relations between total MC concentration and environmental variables showed trends that were not linearized through transformation. Microcystin was undetectable across the range of all variables, and the shape of any given relation was defined by the upper limits created by MC maxima. Nonlinear interval-maxima regression (IMR) (Blackburn *et al.* 1992, Scharf *et al.* 1998) was used to define the shape of these upper limits after Graham *et al.* (2004). Each independent variable was divided into equal increments, resulting in 10–16 intervals. The maximum MC concentration and the associated environmental variable value were obtained from each interval and used in nonlinear regression analysis (Blackburn *et al.* 1992, Scharf *et al.* 1998). Microcystin detection frequency in each interval was calculated by dividing the number of detections in the interval by the total number of samples (n = 1402). We performed IMR using SigmaPlot® (10.0) and set significance at $p < 0.05$.

# Results

## *Descriptive limnology*

Missouri reservoirs encompass a broad range of trophic conditions (Jones *et al.* 2008a; Table 1). Most (79%) reservoirs sampled in 2004–2006 were mesotrophic or eutrophic, with 7% oligotrophic and 14% hypereutrophic. Based on TN:TP

**Table 1.-** Provincial medians (med) and ranges of landscape, morphological, and limnological variables.

| | Ozark Highlands | | | Ozark Border | | | Osage Plains | | | Glacial Plains | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **n** | **Med** | **Range** | **n** | **Med** | **Range** | **n** | **Med** | **Range** | **n** | **med** | **Range** |
| Landscape | | | | | | | | | | | | |
| Watershed area (Ha) | 29 | 5,309 | 155–1,544,940 | 25 | 502 | 50–38,632 | 20 | 2,611 | 253–4,631,671 | 103 | 677 | 25–1,503,965 |
| % Urban cover | 29 | 0 | 0–6 | 25 | 0 | 0–47 | 20 | 2 | 0–77 | 103 | 2 | 0–32 |
| % Forest cover | 29 | 66 | 28–94 | 25 | 40 | 0–82 | 20 | 10 | 0–31 | 103 | 12 | 0–84 |
| % Crop cover | 29 | 2 | 0–11 | 25 | 10 | 0–52 | 20 | 24 | 7–59 | 103 | 28 | 0–70 |
| % Grass cover | 29 | 24 | 1–59 | 25 | 34 | 0–74 | 20 | 46 | 9–73 | 103 | 39 | 1–78 |
| Morphological | | | | | | | | | | | | |
| Mean depth (m) | 29 | 3.7 | 1.8–12.3 | 25 | 3.4 | 1.4–9.8 | 20 | 3.2 | 1.0–9.6 | 103 | 2.7 | 1.1–8.5 |
| Reservoir area (Ha) | 29 | 96 | 6–51,334 | 25 | 46 | 7–379 | 20 | 109 | 14–53,814 | 103 | 40 | 5–18,524 |
| Reservoir volume (Ha m) | 29 | 1,900 | 80–2,020,000 | 25 | 796 | 60–13,782 | 20 | 1,400 | 57–1,202,700 | 103 | 450 | 10–500,040 |
| Flushing rate (Yrs) | 29 | 1.75 | 0.23–87 | 25 | 0.66 | 0.22–42 | 20 | 1.5 | 0.43–6.0 | 103 | 0.73 | 0.03–7.0 |
| Limnological | | | | | | | | | | | | |
| Temperature (°C) | 245 | 26.6 | 16.3–33.5 | 127 | 27.6 | 16.8–33.5 | 140 | 26.3 | 19.5–32.3 | 890 | 26.2 | 14.2–34.3 |
| Dissolved oxygen (mg/L) | 245 | 6.0 | 2.7–11.5 | 127 | 6.0 | 1.5–11.6 | 140 | 5.3 | 1.8–9.7 | 890 | 6.9 | 2.1–15.3 |
| Secchi (m) | 245 | 2.1 | 0.4–7.9 | 127 | 1.1 | 0.2–4.8 | 140 | 0.8 | 0.09–2.5 | 890 | 1.0 | 0.01–7.1 |
| Total nitrogen (mg/L) | 245 | 380 | 40–1,050 | 127 | 640 | 270–2,230 | 140 | 870 | 380–1,810 | 890 | 840 | 240–3,120 |
| Total phosphorus ($\mu$g/L) | 245 | 14 | 3–67 | 127 | 36 | 5–257 | 140 | 49 | 20–237 | 890 | 41 | 6–346 |
| TN:TP | 245 | 25.6 | 5.7–180 | 127 | 18.7 | 5.0–78.3 | 140 | 17.4 | 4.8–35.6 | 890 | 19.8 | 4.5–66.2 |
| Chlorophyll ($\mu$g/L) | 245 | 6 | 1–70 | 127 | 15 | 2–265 | 140 | 27 | 4–185 | 890 | 18 | 1–342 |
| Chl:TP | 245 | 0.4 | 0.08–1.16 | 127 | 0.4 | 0.02–2.0 | 140 | 0.5 | 0.03–1.4 | 890 | 0.4 | 0.01–2.2 |
| Nonvolatile solids (mg/L) | 245 | 1.1 | 0.07–9.6 | 127 | 1.9 | 0–14.3 | 140 | 3.6 | 0.6–22.0 | 890 | 3.0 | 0–42.8 |
| Volatile solids (mg/L) | 245 | 1.4 | 0.3–9.8 | 127 | 2.6 | 0.3–21.6 | 140 | 4.3 | 1.0–17.0 | 890 | 3.5 | 0.2–37.6 |
| Total solids (mg/L) | 245 | 2.7 | 0.6–14.6 | 127 | 4.7 | 0.9–28.8 | 140 | 8.4 | 2.1–38.0 | 890 | 7.1 | 0.3–51.2 |
| Conductivity ($\mu$S) | 245 | 168 | 25–368 | 127 | 118 | 16–489 | 140 | 193 | 67–401 | 890 | 186 | 24–613 |
| Turbidity (NTU) | 245 | 2.1 | 0.5–19.4 | 127 | 3.9 | 0.8–36.3 | 140 | 6.3 | 1.9–80.2 | 890 | 5.2 | 0.5–126 |
| Dissolved organic carbon (mg/L) | 245 | 3.5 | 1.0–7.0 | 127 | 5.5 | 3.0–11.9 | 140 | 5.4 | 3.3–7.8 | 890 | 6.6 | 3.9–12.4 |

Note: For landscape and morphological variables n indicates the number reservoirs; for limnological variables n indicates the number of measurements.

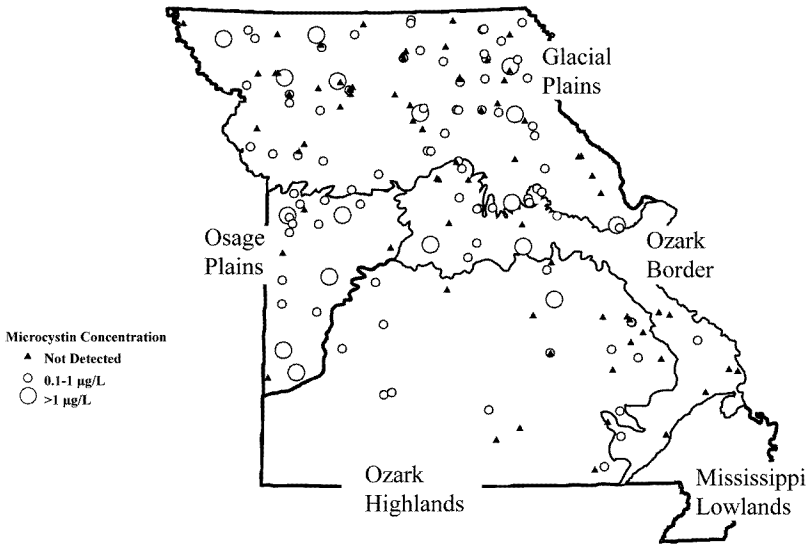


**Figure 1.**-Physiographic location of reservoirs sampled in Missouri and total microcystin occurrence and concentration.

ratios (4–180), potential phosphorus limitation (TN:TP > 17) or co-limitation (17 < TN:TP > 10) by phosphorus and nitrogen (Forsberg and Ryding 1980) was common (93% of samples). Most (88%) samples had Chl:TP ratios substantially <1, suggesting bloom conditions were uncommon (White *et al.* 1985; Table 1).

Regional patterns in limnology matched those described by Jones *et al.*(2008a), with the greatest nutrient and Chl values and the shallowest Secchi depths occurring in Osage and Glacial Plains reservoirs (Table 1). Median nutrient and Chl values in the Plains were 2–3 times greater than in the Ozark Highlands, and the Ozark Border was intermediate; median Secchi depth in the Ozark Highlands was 2 times greater than observed in other physiographic sections, including the Ozark Border (Table 1).

## *Microcystin occurrence and concentration*

Overall, 58% of Missouri reservoirs (n = 177) and 23% of samples (n = 1402) had detectable MC (Fig. 1 and 2). Total MC concentrations ranged from 0.1 to 21 µg/L but were usually <1.0 µg/L (Table 2; Fig. 1 and 2). Few Missouri reservoirs (10%) had MC concentrations exceeding the World Health Organization (WHO) finished drinking-water guideline of 1 µg/L (Table 2; Fig. 1 and 2), and the WHO recreational guideline of 20 µg/L (Chorus and Bartram 1999) was exceeded once during our study. Of the 36 reservoirs sampled for CYL during late summer 2006, 14% had low detectable levels (0.12–0.81 µg/L).

Occurrence and concentration of MC were greatest in eutrophic Osage and Glacial Plains reservoirs (Table 2; Fig. 1). It occurred most frequently in Osage Plains reservoirs (80% of reservoirs, 34% of samples), although the greatest concentrations were collected from Glacial Plains reservoirs (mean = 0.32 µg/L, max = 21 µg/L; Table 2). Despite general regional patterns, MC values >1 µg/L were observed throughout Missouri, with the exception of the southern Ozark Highlands (Table 2; Fig. 1).

Microcystin occurred in Missouri reservoirs throughout summer with mean, median and maximum concentrations being similar across summer months during the 3 summer seasons sampled for this study; however, MC was detected about twice as often in June through August compared to May (Table 3). Occurrence and concentration of MC were similar across years. Between 42 and 52% of reservoirs and 20–26% of samples had detectable MC each summer (Table 4). Mean and median MC concentrations were similar among summers, though 2006 maxima were an order of magnitude less than the others (Table 4). Of the 83 reservoirs sampled during 2 or 3 summers, 60% had detectable MC in more than one summer. Within this group, 11 reservoirs had MC maxima ≥ 1 µg/L and 8 (73%) had detectable MC each summer of sample collection, indicating frequent MC occurrence in some reservoirs.

## *Relation with environmental variables*

We detected MC in reservoirs of all trophic status but occurrence and concentration increased across the trophic gradient: 33% of oligotrophic (n = 12), 45% of mesotrophic (n = 40), 60% of eutrophic (n = 100), and 80% (n = 25) of hypereutrophic reservoirs had detectable MC. Maximum concentrations in eutrophic and hypereutrophic reservoirs were 2 orders of magnitude greater than in oligotrophic reservoirs

**Table 2.**-Regional means, medians, and maxima of total microcystin values. Summary statistics are based on samples collected in each region.

| | | | Total microcystin ($\mu$g/L) | | | | |
|---|---|---|---|---|---|---|---|
| **Region** | **Reservoir n** | **% Detection** | **n** | **% Detection** | **Mean** | **Median** | **Maximum** |
| Ozark Highlands | 29 | 52 | 245 | 11 | <0.1 | <0.1 | 1.2 |
| Ozark Border | 25 | 44 | 127 | 20 | 0.15 | <0.1 | 3.1 |
| Osage Plains | 20 | 80 | 140 | 34 | 0.19 | <0.1 | 4.9 |
| Glacial Plains | 103 | 58 | 890 | 25 | 0.32 | <0.1 | 21 |

Note: Reservoir n indicates the number of reservoirs in each region. n indicates the number of samples collected in each region.

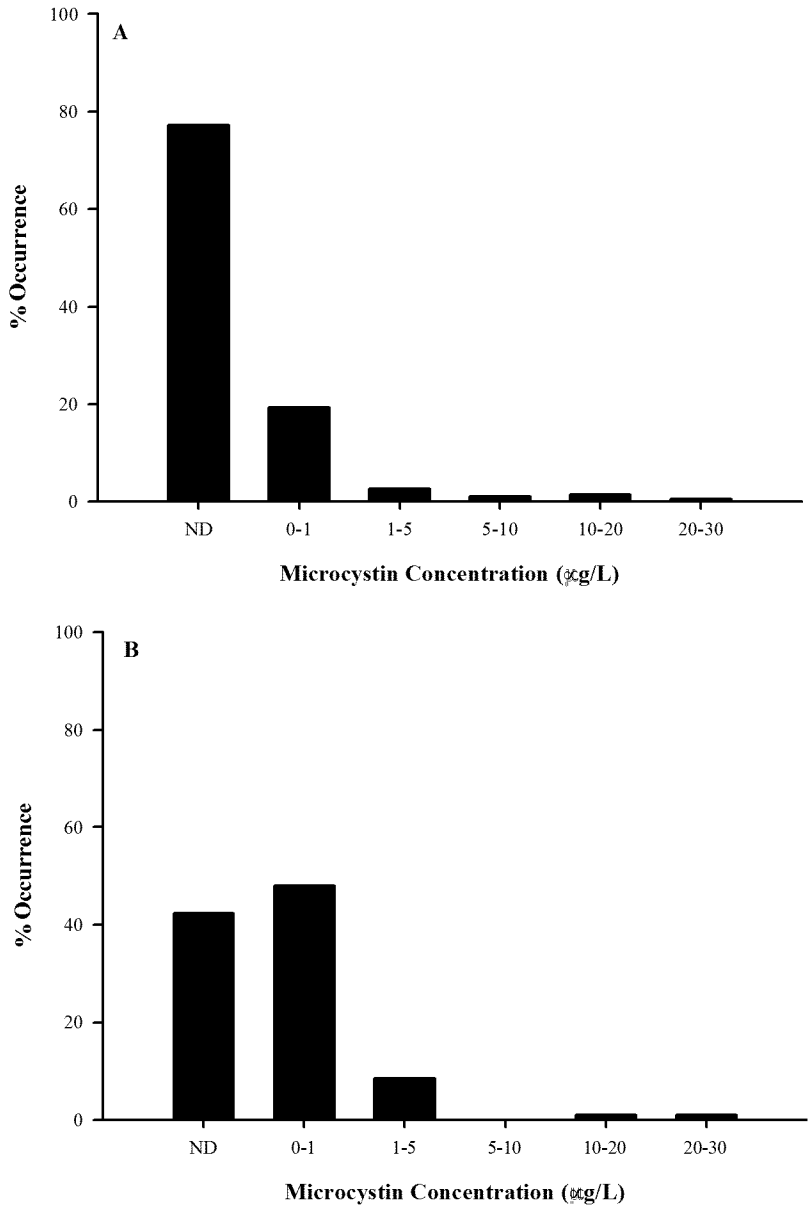


**Figure 2.**-Occurrence and concentration of total microcystin in Missouri reservoirs. The World Health Organization (WHO) microcystin guidelines for finished drinking water and full-body contact recreation are 1 and 20 $\mu$g/L, respectively. (A) Occurrence and concentration among samples (n = 1402), (B) occurrence and concentration among reservoirs (n = 177).

**Table 3.-** Monthly means, medians, and maxima of total microcystin values. Summary statistics are based on samples collected during each month.

| | | | Total microcystin (μg/L) | | | | |
|---|---|---|---|---|---|---|---|
| Month | Reservoir n | % Detection | n | % Detection | Mean | Median | Maximum |
| May | 153 | 18 | 281 | 12 | 0.14 | <0.1 | 13 |
| June | 176 | 40 | 464 | 24 | 0.33 | <0.1 | 21 |
| July | 177 | 35 | 464 | 20 | 0.28 | <0.1 | 11 |
| August | 148 | 35 | 381 | 22 | 0.18 | <0.1 | 4.6 |

Note: Reservoir n indicates the number of reservoirs sampled each month. n indicates the number of samples collected during each month.

and one order of magnitude greater than in mesotrophic reservoirs (Table 5).

As indicated by occurrence and concentration across the trophic gradient (Table 5), nutrient status, Chl content and transparency differed among the 3 MC concentration categories (Table 6). Mean nutrient and Chl concentrations were greatest in the MC >1 μg/L category and smallest in the nondetect category; the MC 0.1–1 μg/L category was intermediate. Total N and TP significantly increased by ~ 2-fold across the MC categories, and Chl by ~ 4-fold (Table 6). Secchi transparency showed the opposite pattern. Mean values decreased significantly by ~ 2-fold across the MC categories (Table 6). Mean TN:TP ratios were significantly greater in the nondetect and MC 0.1–1 μg/L categories (19–20) than the >1 μg/L category (15), although the difference is not as marked as observed for other variables (Table 6). Several landscape and morphological variables also differed among the 3 MC concentration categories, likely reflecting effects on reservoir nutrient values (Jones *et al.* 2004, 2008b).

Despite increases in occurrence and concentration with elevated nutrients and reduced transparency (Table 5 and 6), correlations between MC and trophic state variables were weak (TN: $r = 0.31$, TP: $r = 0.26$, Secchi: $r = -0.19$; all $n = 1402$ and all $p < 0.01$). Correlations of MC concentration with other measured variables in Table 1 also were weak (all $r \leq 0.35$, data not shown). Similar to Graham *et al.* (2004), MC-TN and MC-TP IMR maxima in Missouri reservoirs were characterized by unimodal curves ($r^2 = 0.87$ and 0.75, respectively; both $p < 0.01$), and MC-Secchi IMR

**Table 4.-** Annual means, medians, and maxima of total microcystin values. Summary statistics are based on samples collected during each year.

| | | | Total microcystin (μg/L) | | | | |
|---|---|---|---|---|---|---|---|
| Year | Reservoir n | % Detection | n | % Detection | Mean | Median | Maximum |
| 2004 | 76 | 42 | 514 | 23 | 0.34 | <0.1 | 21 |
| 2005 | 95 | 52 | 380 | 26 | 0.25 | <0.1 | 11 |
| 2006 | 127 | 46 | 508 | 20 | 0.16 | <0.1 | 4.9 |

Note: Reservoir n indicates the number of reservoirs sampled each year. n indicates the number of samples collected during each year.

**Table 5.-** Trophic class means, medians, and maxima of total microcystin values. Summary statistics are based on samples collected in each trophic class.

| | | | Total microcystin (μg/L) | | | | |
|---|---|---|---|---|---|---|---|
| Trophic class | Reservoir n | % Detection | n | % Detection | Mean | Median | Maximum |
| Oligotrophic | 12 | 33 | 94 | 5 | <0.1 | <0.1 | 0.33 |
| Mesotrophic | 40 | 45 | 374 | 10 | <0.1 | <0.1 | 3.1 |
| Eutrophic | 100 | 60 | 776 | 27 | 0.30 | <0.1 | 21 |
| Hypereutrophic | 25 | 80 | 158 | 42 | 0.51 | <0.1 | 10 |

Note: Reservoir n indicates the number of reservoirs sampled in each trophic class. n indicates the number of samples collected in each trophic class.

**Table 6.-** Comparison of environmental variables among three microcystin concentration categories: no detectable microcystin (nd), total microcystin concentrations between 0.1–1 μg/L (0.1–1), and total microcystin concentrations greater than 1 (>1). Significant differences between means were determined using one-way ANOVA and Tukey's pairwise comparisons.

| Variable | n | Mean | Range | F-value | p-value |
|---|---|---|---|---|---|
| Landscape | | | | | |
| % Forest cover | | | | | |
| nd | 1,071 | 22[a] | 0–94 | 24.13 | <0.01 |
| 0.1–1 | 270 | 12[b] | 0–92 | | |
| >1 | 49 | 8[b] | 1–56 | | |
| % Crop cover | | | | | |
| nd | 1,071 | 10[a] | 0–78 | 35.27 | <0.01 |
| 0.1–1 | 270 | 23[b] | 0–78 | | |
| >1 | 49 | 28[b] | 17–78 | | |
| % Grass cover | | | | | |
| nd | 1,071 | 35[a] | 1–78 | 7.26 | <0.01 |
| 0.1–1 | 270 | 39[b] | 2–78 | | |
| >1 | 49 | 44[b] | 17–78 | | |
| Morphological | | | | | |
| Mean depth (m) | | | | | |
| nd | 1,070 | 3.4[a] | 1.1–12.3 | 5.73 | <0.01 |
| 0.1–1 | 266 | 3.2[a] | 1.1–12.3 | | |
| >1 | 46 | 2.6[b] | 1.6–4.2 | | |
| Limnological | | | | | |
| Secchi (m) | | | | | |
| nd | 1,080 | 1.2[a] | 0.01–7.9 | 26.93 | <0.01 |
| 0.1–1 | 271 | 0.9[b] | 0.2–6.8 | | |
| >1 | 49 | 0.7[c] | 0.2–3.5 | | |
| Total nitrogen (μg/L) | | | | | |
| nd | 1,082 | 646[a] | 40–3070 | 77.05 | <0.01 |
| 0.1–1 | 271 | 891[b] | 140–3120 | | |
| >1 | 49 | 1170[c] | 450–2230 | | |
| Total phosphorus (μg/L) | | | | | |
| nd | 1,082 | 32[a] | 3–251 | 51.33 | <0.01 |
| 0.1–1 | 271 | 47[b] | 6–346 | | |
| >1 | 49 | 76[c] | 14–237 | | |
| TN:TP | | | | | |
| nd | 1,082 | 20[a] | 4.5–180 | 9.48 | <0.01 |
| 0.1–1 | 271 | 19[a] | 5.5–67 | | |
| >1 | 49 | 15[b] | 5.6–44 | | |
| Chlorophyll (μg/L) | | | | | |
| nd | 1,082 | 11[a] | 1–342 | 94.34 | <0.01 |
| 0.1–1 | 271 | 26[b] | 1–306 | | |
| >1 | 49 | 46[c] | 3–140 | | |
| Chlorophyll: Total phosphorus | | | | | |
| nd | 1,082 | 0.4[a] | 0.01–2.2 | 70.99 | <0.01 |
| 0.1–1 | 271 | 0.6[b] | 0.06–1.6 | | |
| >1 | 49 | 0.6[b] | 0.2–1.4 | | |
| Volatile suspended solids (mg/L) | | | | | |
| nd | 1,073 | 2.6[a] | 0.2–25 | 84.86 | <0.01 |
| 0.1–1 | 267 | 4.4[b] | 0.2–38 | | |
| >1 | 49 | 7.6[c] | 0.6–19 | | |
| Total suspended solids (mg/L) | | | | | |
| nd | 1,072 | 5.4[a] | 0.3–51 | 37.35 | <0.01 |
| 0.1–1 | 267 | 7.2[b] | 0.7–51 | | |
| >1 | 49 | 11.5[c] | 1.0–39 | | |

*(Countinued on next page)*

**Table 6.**- Comparison of environmental variables among three microcystin concentration categories: no detectable microcystin (nd), total microcystin concentrations between 0.1–1 μg/L (0.1–1), and total microcystin concentrations greater than 1 (>1). Significant differences between means were determined using one-way ANOVA and Tukey's pairwise comparisons. (Continued)

| Variable | n | Mean | Range | F-value | p-value |
|---|---|---|---|---|---|
| Turbidity (NTU) | | | | | |
| nd | 1081 | 4.0[a] | 0.5–126 | 56.53 | <0.01 |
| 0.1–1 | 271 | 5.7[b] | 0.7–44 | | |
| >1 | 49 | 11.5[c] | 1–48 | | |
| Dissolved organic carbon (mg/L) | | | | | |
| nd | 1082 | 5.4[a] | 1.0–11.9 | 44.40 | <0.01 |
| 0.1–1 | 271 | 6.4[b] | 1.4–12.4 | | |
| >1 | 49 | 7.6[c] | 3.8–11.4 | | |

[a,b,c]Indicate significant differences in mean values for each variable. Table 1 variables without significant differences ($p > 0.05$) are not shown.



maxima were characterized by exponential decline ($r^2 = 0.93$, $p < 0.01$; Fig. 3). Frequency distributions of MC occurrence along these environmental gradients showed similar patterns. Along the TN gradient the greatest MC values (>10 μg/L) occurred between 600 and 1900 μg/L; for TP, MC peaked between 30 and 170 μg/L, ranges indicative of eu-hypereutrophic conditions in Missouri reservoirs (Jones *et al.* 2008a; Fig. 3). Microcystin >10 μg/L occurred at Secchi depths <2 m, encompassing meso-hypereutrophic conditions (Fig. 3). The most frequent detection of MC occurred within narrower environmental ranges. We detected MC most frequently at TN between 600 and 1,200 μg/L (eutrophic conditions), TP between 15 and 70 μg/L (meso-eutrophic conditions) and Secchi depths between 0.5 and 1.0 m (eutrophic conditions), a result likely due in part to the large number of samples collected within these ranges (Fig. 3).

## Discussion

Cyanobacteria frequently dominate summer algal biomass in Missouri reservoirs, and *Anabaena* is the most commonly occurring potential MC producer (Jones *et al.* 2008a). Microcystin was common in Missouri reservoirs, but few concentrations were large enough to cause human health concerns (Fig. 2). Occurrence and concentrations were similar across years, with approximately 50% of reservoirs sampled having detectable MC each summer (Table 4). Widespread occurrence with relatively few large values is characteristic of regional MC studies worldwide (Chorus and Bartram 1999, Graham *et al.* 2004, Boyer 2007, Williams *et al.* 2007, Hedman *et al.* 2008), a consequence of extreme temporal and spatial variability in MC concentrations (Lindholm 1991, Kotak *et al.* 1995, Welker *et al.* 2003). Among reservoirs with MC maxima ≥1 μg/L, 73% had detectable MC each summer, indicating seasonal inventory studies can identify reservoirs with potential for toxin production.

Microcystin maxima in Missouri reservoirs (21 μg/L) were orders of magnitude less than cyanobacterial blooms in Wisconsin (7,600 μg/L) and Minnesota (8,400), although such large values are not necessarily typical of Midwestern blooms (Hedman *et al.* 2008, Lindon and Heiskary 2008). The monthly collections from open-water pelagic locations likely missed concentrated surface accumulations of cyanobacteria; therefore, concentrations large enough to cause human-health concerns during recreational activities are likely under-represented (Chorus and Bartram 1999, Graham *et al.* 2008, Lindon and Heiskary 2008). Toxic cyanobacterial blooms have been documented in the Midwestern United States since the late 1800s (Yoo *et al.* 1995), and anecdotal reports of animal illness and death after exposure to cyanobacterial blooms are common throughout the region, including Missouri (Graham 2006).

About one-half of reservoirs in this study had detectable MC, similar to the earlier findings of Graham *et al.* (2004) in Missouri. Graham *et al.* (2004) sampled particulate MC using a 64-μm mesh plankton net, which may enhance detections but under-represent total concentrations by loss of algal material through the net (Graham and Jones 2007). Maximum MC concentrations were ~3 μg/L in the Graham *et al.* (2004) study, compared to 21 μg/L in this dataset. These MC data come from whole-water samples, which better represent the entire cyanobacterial community and more accurately estimate MC concentration (Graham and Jones 2007).

Metalimnetic populations of potential MC producing cyanobacteria occasionally bloom at depth, particularly in mesotrophic systems (Lindholm 1991, Graham *et al.* 2008). Collection of surface samples will miss detection of these subsurface blooms and may result in underestimates of toxin occurrence. In Missouri reservoirs, there is typically little difference in Chl values at the surface and at the Secchi depth, indicating subsurface Chl peaks are uncommon (Jones *et al.* 2008a); therefore, subsurface cyanobacterial blooms likely do not represent a large proportion of microcystin occurrences in Missouri reservoirs.

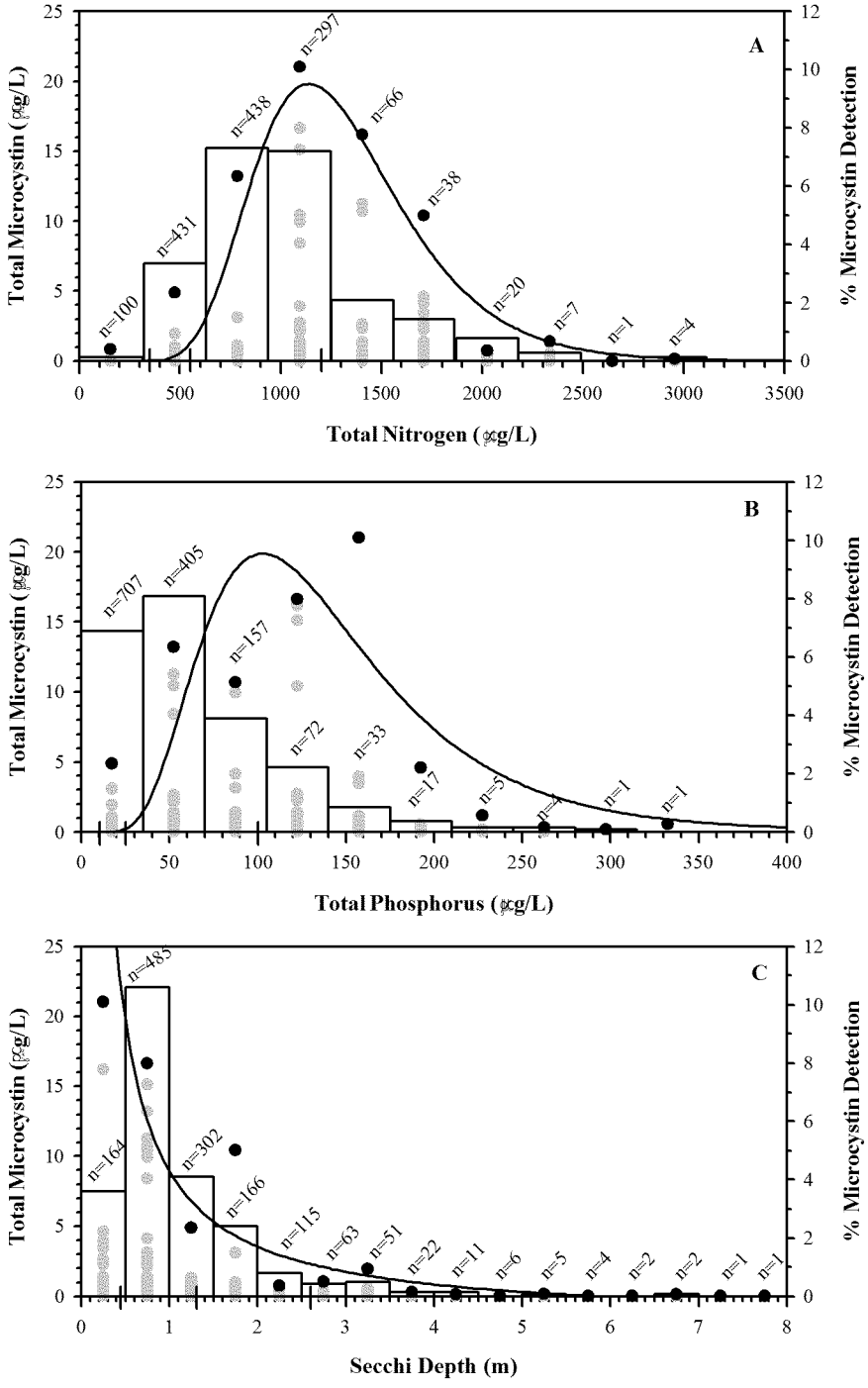


**Figure 3.-**Total microcystin (MC), total nitrogen (TN), total phosphorus (TP) and Secchi bivariate relations. Data shown as grouped into intervals for interval-maxima regression (IMR). Curves were estimated using IMR. Black points indicate the data used for IMR analysis (n = 8–16) and gray points indicate all other data. Bar graphs represent frequency distributions of MC detection within each interval used for IMR. Hash marks on the x-axes indicate oligotrophic/mesotrophic, mesotrophic/eutrophic and eutrophic/hypereutrophic cutpoints for Missouri reservoirs as defined by Jones *et al.* 2008a. (A) MC–TN relation ($r^2$ = 0.87, $p < 0.01$, values for line fitted to black points only), (B) MC–TP relation ($r^2$ = 0.75, $p < 0.01$), (C) MC–Secchi relation ($r^2$ = 0.93, $p < 0.01$). All n = 1402.

This is the first report of CYL in Missouri. Potential CYL producers, including *Anabaena*, *Aphanizomenon* and *Cylindrospermopsis*, can dominate summer algal assemblages in Missouri reservoirs (Graham *et al.* 2006a, Jones *et al.* 2008a), but the toxin was detected in few samples and always at concentrations <1 μg/L. In most of the United States, CYL occurs relatively infrequently (<10% of samples) and when detected usually at concentrations <1 μg/L (Boyer 2007, Hedman *et al.* 2008), although in Florida CYL has occurred in up to 30% of samples (Williams *et al.* 2007) and at concentrations as large as 200 μg/L (Burns 2008).

In temperate climates, toxic cyanobacterial blooms historically have occurred most frequently during late summer–early fall (Chorus and Bartram 1999, Huisman *et al.* 2005); however, in this study, there were no distinct patterns during summer, and MC maxima were observed anytime between May and August (Table 3). Similarly, MC concentrations of concern for recreational exposure were observed from May to September in 12 eutrophic Minnesota lakes (Lindon and Heiskary, 2008). Microcystin maxima may have occurred during months other than May–August in Missouri reservoirs. Previous studies in Missouri have documented year-round MC occurrence, and peak concentrations occasionally occur during winter (Graham *et al.* 2006a, 2006b). Thus, MC is a potential recreational concern throughout summer and a potential drinking water concern year-round in Midwestern reservoirs and lakes.

Missouri reservoirs with the greatest MC values had increased nutrients and a reduced light environment (Table 6; Fig. 3), characteristics conducive to cyanobacterial dominance (Chorus and Bartram 1999). General patterns in MC with respect to trophic state match these patterns, with peak occurrence and concentrations in eutrophic to hypereutrophic reservoirs (Table 5; Fig. 3). Even so, MC occurred in reservoirs of all trophic status and large nutrient values and shallow Secchi depths were not consistently related to elevated MC values (Tables 5 and 6; Fig. 3). Because of co-occurrence of toxic and nontoxic cyanobacterial strains (Vézie *et al.* 1998) and the range of factors that may drive cyanobacterial community dynamics (Reynolds 1998), regional relations between environmental variables and MC occurrence and concentration are invariably complex (Kotak *et al.* 2000, Chorus 2001, Graham *et al.* 2004).

Nonlinear relations that describe MC maxima along gradients of nutrients and light (Fig. 3) were similar to those described by Graham *et al.* (2004) for particulate MC in the Midwest; however, TN and TP ranges where MC maxima were encountered in Missouri are shifted to peak at lower nutrient levels and are narrower than those defined for the region (Fig. 3; Graham *et al.* 2004). Nonlinear interval-maxima regression is limited by the range of MC values and environmental conditions encountered (Blackburn *et al.* 1992, Scharf *et al.* 1998, Graham *et al.* 2004). Concentrations of MC in Missouri were orders of magnitude greater, and TN and TP values orders of magnitude less, than in the midcontinent region sampled by Graham *et al.* (2004). The Midwestern relations are likely more representative of global conditions and are similar to relations described in Germany (Chorus 2001) and Alberta, Canada (Kotak *et al.* 2000). The Missouri relations are local, and may be used to identify conditions under which elevated MC concentrations most frequently occur.

This study demonstrates that MC is common in Missouri reservoirs; most values are low, but concentrations of concern were detected. Though timing of peak concentrations were unique to individual reservoirs, statewide occurrence was relatively consistent among months and years, indicating that including MC as part of routine water-quality monitoring programs may be sufficient to identify reservoirs with frequent MC occurrence; these reservoirs mostly are in the midrange of the trophic continuum. These data enhance understanding of when and where elevated MC concentrations may occur and can lead to more effective identification of human health risks and lake and reservoir management strategies.

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